Implications of a polymer meniscus implant on knee tribology

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Chapter 4

An *in vitro* Study of Cartilage–Meniscus Tribology to Understand the Changes Caused by a Meniscus Implant

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(submitted for publication)

Abstract

Active lifestyles increase the risk of meniscal injury. A permanent meniscus implant of polycarbonate urethane (PCU) is a promising solution to prevent total knee arthroplasty. Study of the changes in articular cartilage tribology in the presence of PCU is essential in developing the optimum meniscus implant. The purpose of this study is to examine changes in cartilage tribology caused by meniscus replacement with a permanent implant. A cartilage–meniscus reciprocating, sliding model was developed mimicking the stance and swing phases of the gait cycle, to evaluate its tribological properties in simulated physiological conditions. The meniscus was replaced with PCU to study the changes. Coefficient of friction (COF) was measured during sliding, and wear of cartilage was determined histologically and quantified according to a custom-made scoring system. In addition to PCU itself, three surface modifications of PCU were evaluated: PCU with C18 chains, with mono-functional PDMS (polydimethylsiloxane) and with mono-functional PTFE (polytetrafluoroethylene) groups. Cartilage–meniscus sliding resulted in low COF during both stance and swing ($0.01 < COF < 0.12$) and low wear of cartilage (scores < 1). Cartilage–PCU sliding, during stance, revealed similar low COFs. But during swing, the COFs were high (~1) with a maximum value of 1.6. COF increased with increasing the sliding time and decreased with increasing the contact pressure (according to a power equation) up to 1 MPa. The tested biomaterials and meniscus occasionally damaged the cartilage. No systematic correlation was found between the damage and the experimental condition. Changes in the lubricant solution or surface modification of PCU did not affect PCU's tribological performance. Therefore, Replacement of native meniscus with PCU resulted in an increase of the COF during the swing phase of the gait cycle, which is indicative of breakdown in interstitial fluid pressurization lubrication and therefore non-effective activation of the boundary lubrication. The wear of cartilage against biomaterial was not higher than its wear against meniscus under experimental conditions. To be concluded, permanent meniscus implants made of PCU, which show high COF during the swing phase of the gait cycle, may result in patient discomfort and wear of cartilage in the long term.

Introduction

Meniscal injuries are associated with active life styles[124] and professions.[125] Age, gender (male) and activity increase the chances of such injuries.[124,126] Of all knee injuries 14.5−38% are meniscal injuries.[124,126] The incidence of sports-related meniscal lesions leading to a meniscectomy is 60−70 per 100,000 patients per year.[124] Additionally, the natural aging process causes degenerative tears of the meniscus.[21]

The medial and lateral menisci in the knee joint are crescent-wedge-shaped fibro-cartilaginous tissues, located in between the weight bearing surfaces of the tibia and femur.[21] Menisci play an essential role in the load distribution and stability of the knee joint. The lateral meniscus is much more mobile as compared to the medial meniscus, which can explain the higher risks of generating medial meniscal tears.[21]

The porous and permeable meniscus tissue consists mainly of water, i.e., 63−75% of the total weight.[127] Two types of cells are recognized in the meniscus, fibro-chondrocytes (in the inner and middle part of the meniscus) surrounded by an extra-cellular matrix (ECM) and fibroblast-like cells (in the outer part of the meniscus) surrounded by a dense connective tissue.[127] The dry weight of the matrix is composed of 75% collagen (90% type I collagen and 10% type II, III and V collagens) and 2.5% proteoglycans (mainly aggrecan).[127] The regenerative capability of the meniscus is limited due to vascularization in only the peripheral one-third of its volume.[21,24]

The menisci rub against a thin layer (1−5 mm)[1] of avascular articular cartilage, which covers the ends of the femoral condyle and tibia plateau. Articular cartilage consists of 70−80% water. Collagens make up to ~60% of the dry weight (90% of which is type II collagen) and proteoglycans ~30%. The final 10% is made up of non-collagenous proteins, chondrocytes and lipids. The chondrocytes at the superficial zone synthesize a superficial zone protein (SZP, also known as proteoglycan 4, PRG4 or lubricin), which plays an important role in the lubrication of the articular cartilage.[9]

Articular cartilage and menisci are surrounded by synovial fluid. Synovial fluid components—hyaluronan (HA), albumin, proteoglycan 4 (PRG4, also known as lubricin) and surface-active phospholipids (SAPL)—absorb in/on the cartilage and meniscus surfaces and provide lubricating properties there.[26]

In a healthy knee joint, articular cartilage, meniscus and synovial fluid, together, provide coefficient of friction (COF) as low as 0.005 as well as excellent

wear protection.[25] A variety of lubrication mechanisms have been proposed to be responsible for this unique tribological system.[28,34,36,82] "Interstitial fluid pressurization and weeping" (IFPW)[34,128] in combination with boundary[39,105] lubrication mechanisms are considered to be active.

A meniscus has a limited capacity to regenerate.[21,24] Untreated tears and consequently loss of function change the load distribution inside the knee, resulting in early signs of degenerative arthritis.[58] Repairing strategies, e.g., sutures, anchors or staples, are only applicable on the vascularized zone of meniscus, even so it is not often reliable.[129] Meniscectomy (partial or total) immediately relieves pain and improves the knee function, yet 50% of the patients show symptoms of premature osteoarthritis.[58] The current treatment for symptomatic patients (post-meniscectomy) is the transplantation of meniscal allografts—from a donor meniscus—which also relieves pain and improves the knee function.[61] The limited availability of allografts of appropriate size, the risk of transmission of disease and the shrinkage of allograft post-implantation are major drawbacks of this approach.[61]

Therefore, developing a meniscus implant is a viable option. Implants based on biodegradable scaffolds, made of natural or synthetic polymers, aiming for the regeneration of the meniscus[65,66,68–71] or permanent synthetic implants replacing the native meniscus[60,72–75] have been developed. An anatomically shaped, polycarbonate urethane medial meniscus implant—reinforced circumferentially with ultrahigh molecular weight polyethylene fibers—(NUsurface® by Active Implants) showed promising results after six months in a sheep model.[60] For human use, the design was changed to a freely floating, disk-shaped implant, which requires the presence of the peripheral rim of the native meniscus. Therefore, it is not an appropriate choice for patients having a total meniscectomy.[77] Preliminary clinical results showed considerable pain relief, although there were major complications due to implant dislocation, fracturing or tearing and inflammation or progression of osteoarthritis.[78,79] Recently, a project (TRAMMPOLIN) was funded by the Dutch BioMedical Materials program to design an anatomically shaped permanent meniscus implant made of polycarbonate urethane.[130]

On the fundamental level, there is still little known about the tribology of cartilage and meniscus or the effects on the knee tribology upon replacing the meniscus with an implant. As opposed to a native meniscus, an artificial meniscus implant, being non-porous, is incapable of contributing to the joint lubrication through IFPW.[34,128] Therefore, once it is implanted, the artificial meniscus can only provide boundary lubrication on its surface with the help of adsorbed synovial fluid molecules.[131]

The first aim of this study was to examine the native cartilage–meniscus tribology in simulated physiological conditions. The second aim was to clarify the tribological changes that occur when the native meniscus is replaced with an artificial one: introduction of a biomaterial (polycarbonate urethane used for NUsurface®) in an otherwise healthy knee joint. The third aim was to speculate the operative lubrication mechanism when an artificial permanent meniscus is implanted in a knee joint and to study whether surface modifications, aimed to enhance boundary lubrication, would improve COF and decrease wear of cartilage. Therefore, a cartilage–meniscus model, with a reciprocating unidirectional movement was developed to measure the friction and wear at the interface. One part of the reciprocating cycle was loaded, mimicking the stance phase of the gait cycle during normal walking, while the other part was low-loaded, mimicking the swing phase. The same model was used to study the cartilage–biomaterial interface and to understand the operative type of lubrication mechanism. The polycarbonate urethane materials with surface modifications were used to see whether the modifications were able to affect the tribology of the cartilage–biomaterial interface.

Experimental Section

Materials. Polycarbonate urethane (Bionate 80A, PCU) and PCUs with surface modifications—Bionate II 80A (mPCU-c, with C18 chains), Bionate 80A S (mPCU-s, with mono-functional PDMS groups) and Bionate 80A 2F (mPCU-f, with mono-functional PTFE groups)—were provided by DSM Biomedical (Geleen, The Netherlands). The samples were injection-molded disks (diameter ≈ 37 mm and thickness ≈ 4 mm; **Figure 1A**). Hyaluronic acid sodium salt (hyaluronan, HA) (average M_w of 3.0 × 10^6 Da) was purchased from Kraeber & Co GMBH, (Ellerbek, Germany). Bovine serum albumin (98−99%) (BSA) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were from Sigma-Aldrich, Ltd. (St. Louis, MO). Bovine proteoglycan 4 (PRG4) was isolated, purified and characterized as described previously.[16] n-Hexane was from Acros Organics (Geel, Belgium). Formaldehyde solution 4% was from Klinipath (Deventer, The Netherlands). Chloroform and Titriplex® III (EDTA, ethylene-di-nitrilotetraacetic acid disodium salt dihydrate) were from Merck (Darmstadt, Germany).

Preparation of POPC vesicles. POPC was first dissolved in chloroform. Then, chloroform was evaporated by blowing filtered nitrogen gas over the solution, completed by 45 min of vacuum drying at room temperature. The dried POPC film was resuspended in 10 mM phosphate-buffered saline (PBS) resulting in a suspension of POPC vesicles of different sizes. To obtain a mono-dispersed, homogeneous vesicle size distribution the suspension was filtered using a mini-extruder

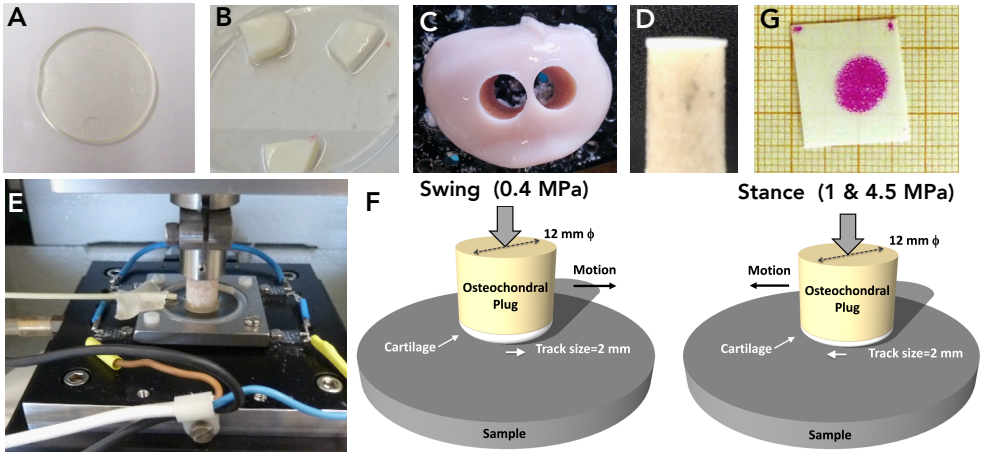


Figure 1. A: PCU disk. **B:** Bovine meniscus samples. **C:** Bovine femoral head with 2 holes from which **D:** osteochondral plugs were harvested. **E:** Experimental set-up (UMT-3). **F:** Schematic illustration of loading and low-loading part of the cycles (stance and swing phases of the gait cycle) during the friction measurements with UMT-3. **G:** Pre-scale film with a contact area mark.

(Avanti Polar Lipids, Inc., Alabaster, USA), in three steps using membranes with decreasing pore diameter—1000, 400 and 100 nm (Whatman, Nuclepore Track-Etch Membrane, St. Louis, MO). The solution was forced through each membrane for 11 times.[132] The size of the vesicles in the suspension (138 ± 24 nm) was measured using light scattering (Zetasizer, Malvern Instruments Ltd., Worcestershire, UK), to assure the mono-dispersity of the solutions.

Preparation of lubricant solutions. Four different solutions in 10 mM PBS were prepared: BSA+HA, BSA+HA+PRG4, BSA+HA+POPC and BSA+HA+PRG4+POPC. The concentrations of the components were: 5 mg/mL BSA,[11] 2 mg/mL HA,[13] 100 μg/mL PRG4[16] and 150 μg/mL POPC[19] to mimic the physiological concentrations in healthy synovial fluid.[105,131] HA molecules were mixed with PBS 24 hours prior to measurements to allow complete dissolution.

Cleaning of polymer disks. All the PCU disks were cleaned with n-hexane, rinsed with ultrapure water (Milli-Q) and then hydrated in sterile ultrapure water for at least 2 weeks prior to experimentation, to allow for swelling.

Preparation of osteochondral plugs and meniscus samples. Bovine stifle joints (~2 year-old and males) were purchased from a local slaughterhouse (Kroon Vlees, Groningen, The Netherlands). The joints were dissected 1 day after slaughter and

delivered intact (unopened with surrounding tissues) and vacuum-packed. To prepare osteochondral plugs and meniscus samples, the joint was opened at room temperature (20−24 °C). All the visibly damaged or arthritic joints were excluded.

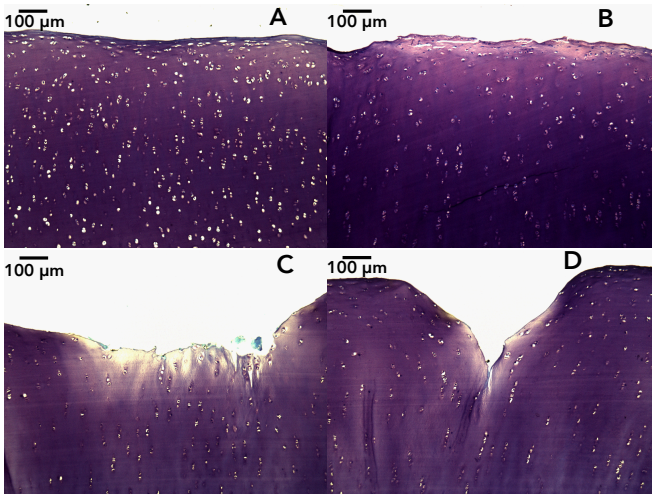
Meniscus samples were cut off from the menisci—from the side in contact with the tibial surface—using a scalpel (sample thickness ≈ 3 mm and surface ≈ 15 × 15 mm^2) (**Figure 1B**). Immediately prior to the test, meniscus samples were fixed onto a silicon rubber slab using pins to stay well-stretched and in-place during the experiment.

Osteochondral plugs of 12 and 6 mm in diameter were drilled out of the femoral condyles (**Figure 1C,D**) using hollow drill bits. The 6 mm plugs were used for cartilage–meniscus as well as for high contact pressure cartilage–PCUs tests. The 12 mm plugs were used for low contact pressure cartilage–PCUs tests.

Sample preparation was done under continuous wetting and cooling with PBS. Touching the surfaces intended for sliding was carefully avoided. Samples were rinsed with PBS and stored in PBS at 4 °C and used at the same day.

Tribology tests. A CETR-UMT-3 (Universal Mechanical Tester) (Bruker Corporation, USA) (**Figure 1E**) was used in a reciprocating configuration to measure the COF —the ratio of the friction force and the applied normal force—at the cartilage–meniscus and cartilage–biomaterial interfaces. An osteochondral plug was mounted at the load cell and slid against meniscus or one of the PCUs at a speed of 4 mm/s and frequency of 1 Hz,[27] at 33 °C—the temperature inside a healthy human knee joint.[133] Experiments were done in one of the indicated lubricant solutions. A specific loading/low-loading condition was used to simulate the gait cycle during normal walking. The load during the low-loaded part of the cycle (swing phase of the gait cycle) was kept constant at 4 N (0.4 MPa), while the load during the loaded part of the cycle (stance phase of the gait cycle) was varied according to the experiment (**Figure 1F**). For experiments longer than 1 hour ultrapure water was added using a peristaltic pump at a flow rate of 0.13 μL/s to compensate for evaporation.

During the first phase COFs were measured at the cartilage–meniscus, cartilage–PCU, cartilage–mPCU-c, cartilage–mPCU-s and cartilage–mPCU-f interfaces, for 1 hour (simulating 9 hours of normal activity), at a P_c of 1 MPa (40 N) during stance, in the presence of one of the solutions, BSA + HA, BSA + HA + PRG4, BSA + HA + POPC or BSA + HA + PRG4 + POPC. During the second phase COFs were measured at the interfaces of cartilage–meniscus, cartilage–PCU, cartilage–mPCU-c, cartilage–mPCU-s and cartilage–mPCU-f, for 4 hours (simulating 36 hours of normal activity), at a P_c of 1 MPa (40 N) during stance, in the solution containing BSA +



1	Surface abrasion	Scores to be given
	None	0
	Minor	1
	Moderate	2
	Severe	3
2	Clefts up to the radial zone	Scores to be given
	None	0
	1–2	4
	≥ 3	5
3	Clefts up to the tidemark	Scores to be given
	None	0
	1	6
	≥ 1	7
	Total Score	Summation of all

Figure 2. Pictures of the histological sections showing the surface of the articular cartilage **A:** without damage on the surface, corresponds to score = 0, **B:** with surface abrasion, corresponds to score = 1–3, in this case a minor damage corresponds to score = 1, **C:** with severe surface abrasion, corresponds to score = 3 and **D:** clefts up to the radial zone, corresponds to score = 4. The table explains of the scoring system in details. No clefts up to the tidemark were observed in this study.

HA + PRG4 + POPC. During the final phase COFs were measured at the interfaces of cartilage–meniscus and cartilage–PCU, for 1 hour, at the P_c of 4.5 MPa (170 N) during stance, in the solution containing BSA + HA + PRG4 + POPC.

A custom-made MATLAB program was used to extract the mean and standard deviation from each cycle for each experiment. Care was taken to only consider COFs that were measured at an applied load within ± 50% of the target load.

Contact area measurement. After each experiment, the contact area between the plug and sample was visualized using pre-scale films (Extreme Low Pressure 4LW, Fujifilm, Tokyo, Japan) by inserting the film in between the cartilage and meniscus/biomaterial (**Figure 1G**) and then applying the desired load. The contact area and aspect ratio (the ratio between the major and minor axes) were determined using a custom-made MATLAB program. The contact area was used to calculate the contact pressure (P_c) of each experiment.

Histology. All the osteochondral plugs were prepared for histological examinations to assess damage of the cartilage during the friction tests. The plugs were fixated in 4% paraformaldehyde solution for at least 48 hours, then rinsed thoroughly with running water and once with PBS. Most of the bony part of the plugs

was removed before the remaining subchondral bone was decalcified using 10% EDTA (10% Titriplex in ultrapure water) for at least 2 weeks while being shaken. EDTA solution was refreshed every 2–3 days. The samples were then dehydrated in a series of ethanol solutions, cleared in xylol and embedded in paraffin. Sections of 5–7 µm were cut using a microtome (Leica RM2235, Leica Microsystems, Rijswijk, The Netherlands). Three slides with sections of each sample were dewaxed, rehydrated and stained with thionine, hematoxylin/eosin and Syrius Red, respectively, and studied using a light microscope.

Quantifying the cartilage wear. Cartilage damage was quantified using a custom-made scoring system explained in the inset table of **Figure 2** which related to cartilage damage as shown in **Figure 2**. The damage was scored in three categories: (1) surface abrasion, (2) clefts up to the deep zone and (3) clefts up to the tidemark, and the final score is the summation from the three categories. Scoring was performed by two evaluators (SEM, RK), which were blinded for the experimental settings.

Statistical analysis. Unpaired, two-tailed Student's t-test was performed to assess the significance of differences between groups. Differences were deemed significant if $p \leq 0.05$. Histological scores were analyzed using a Kruskal-Wallis one way analysis of variance on ranks. If appropriate a Dunn's post hoc test was used for differences between individual samples.

Results

Friction

– *Correlation between the Stance and Swing Phases*

Figure 3A shows a typical plot of COF versus cycle number measured during the first phase of the experiments (explained in experimental section) between the interfaces of cartilage–meniscus (control) and cartilage–biomaterial. At the cartilage–meniscus interface, COF remained low and was similar during both stance and swing (**Figure 3A**, black lines). At the cartilage–biomaterial interface, on the other hand, the COF was low only during stance, during swing the COF increased with increasing cycle number (**Figure 3A**, blue lines).

In the experimental setup, each loaded part of the cycle (stance) was followed by the low-loaded part (swing) and vice versa. Thus, the interfacial changes occurring during one part of the cycle affect the other part as well. **Figure 3B** shows that if the swing COF increased after 1-hour measurement, the stance COF increased linearly too. For the cartilage–PCUs interface, the COF at stance remained

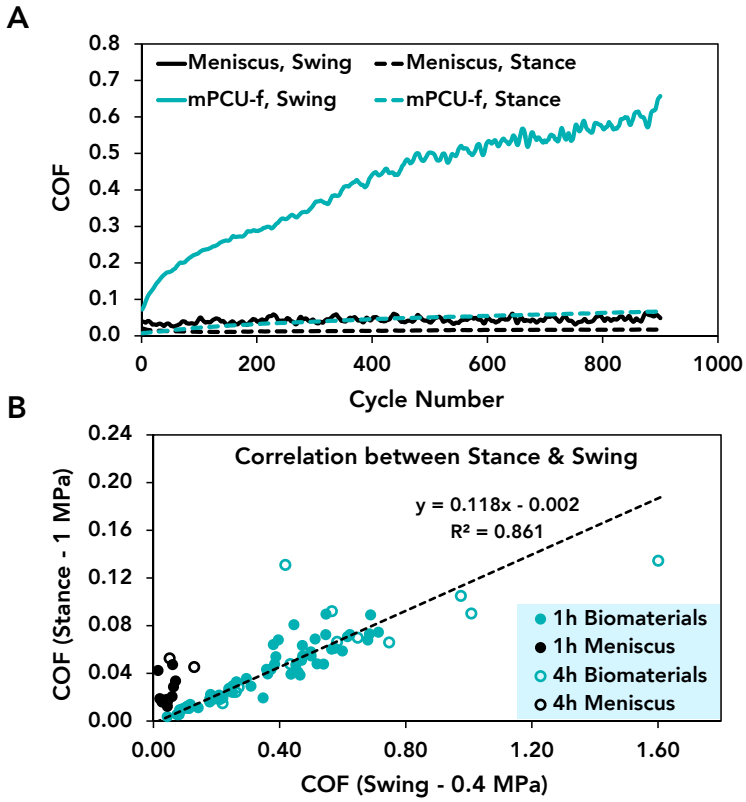


Figure 3. A: COF versus cycle number measured at the cartilage–meniscus (black lines) and cartilage–mPCU-f (blue lines) interfaces, in the presence of BSA+HA+POPC in PBS, for 1 hour, at 1 MPa during stance (dashed lines) and 0.4 MPa during swing (solid lines). **B:** The COF during the last cycle of each measurement was plotted to show the correlation between the COF of the stance (1 MPa) versus the swing (0.4 MPa), for meniscus (black) and all the PCU materials (blue), measured in the presence of all the lubricant solutions, for 1 hour (filled circles) and measured in the presence of BSA+HA+PRG4+POPC, for 4 hours (empty circles).

~one-tenth of the COF at swing (0.118 times lower). At the cartilage–meniscus interface, the COFs at stance and swing did not follow the same linear relation. **Figure 3B** shows that during the first 1 hour of swing, COF at the cartilage–meniscus interface (control) was lower than 0.07 (with a minimum value of 0.01), whereas the COF at the cartilage–PCUs interface increased up to 0.71. The COF at stance remained below 0.05 and 0.09, for the cartilage–meniscus and cartilage–PCUs interfaces, respectively.

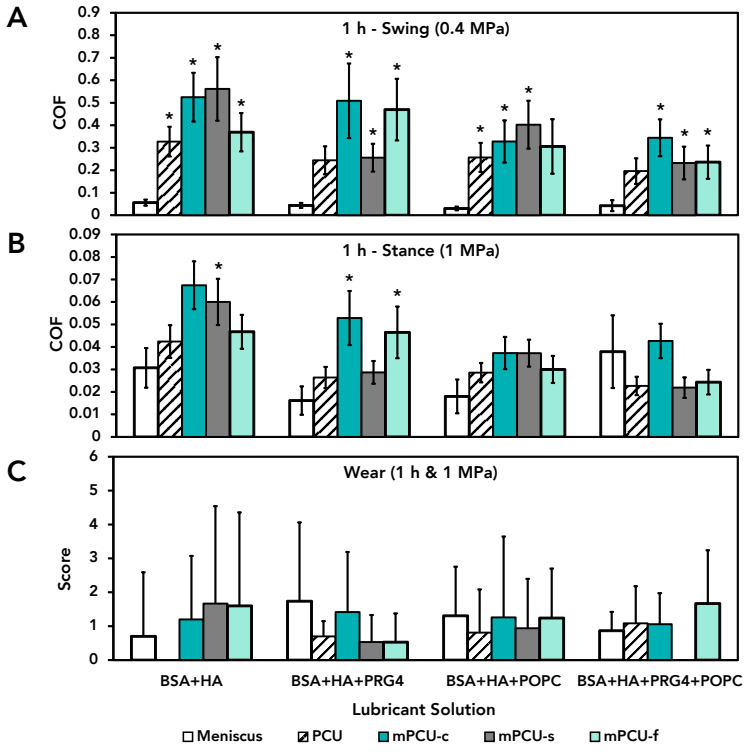


Figure 4. Average COF measured in the presence of different lubricant solutions during **A:** swing (0.4 MPa) and **B:** stance (1 MPa), in 1-hour experiments, on PCU material, with different surface modifications (plots **A** and **B** have different scales). Error bars indicate SD of the mean of COFs over 2–6 separate measurements. **C:** Scores of wear of cartilage at the end of the friction experiments reported in **A** and **B**. Error bars indicate SD of the mean of the scores. Asterisks (*) denote significant differences ($p < 0.05$) as compared to meniscus in that specific solution.

– Effect of Surface Modification and Lubricant Molecules

Average COF at the cartilage–meniscus interface (control), including stance and swing, remained low, 0.06 ± 0.01, irrespective of the lubricant used. The lowest measured COFs were 0.016 ± 0.006 and 0.030 ± 0.008 and the highest measured COFs were 0.037 ± 0.016 and 0.050 ± 0.013 during stance and swing phases, respectively (**Figure 4A,B**).

Replacing meniscus with PCU significantly increased the COF only during swing in solutions containing BSA+HA ($p = 0.039$) and BSA+HA+POPC ($p = 0.021$) (**Figure 4A**). The maximum COF measured at the cartilage–PCU interface in 1 hour was 0.33 ± 0.07.

Replacing meniscus tissue with mPCU-c, mPCU-s and mPCU-f significantly increased the COF during swing in all lubricant solutions. The only exception was mPCU-f in BSA+HA+POPC (p = 0.060) (**Figure 4A**). The highest COF (0.56 ± 0.14) was measured at the cartilage–mPCU-s interface, in the presence of BSA+HA. During stance, replacing meniscus with mPCU-s in BSA+HA (p = 0.051) solution or mPCU-c (p = 0.005) and mPCU-f (p = 0.021) in BSA+HA+PRG4 solution significantly increased the COF (**Figure 4B**). All the PCU materials behaved as well as meniscus in BSA+HA+POPC and BSA+HA+PRG4+POPC, during stance (**Figure 4B**).

Comparing all PCU materials, the lowest average COF was found with PCU, 0.023 ± 0.004, in BSA+HA+PRG4+POPC (the most complete lubricant solution) (**Figure 4B**). Comparing individual measurements, then the lowest measured COF at cartilage–biomaterial interface was 0.0035 which was 3.5 fold lower than 0.012, the lowest measured COF at a cartilage–meniscus interface.

No significant differences were found between modified and unmodified PCUs with respect to the COFs measured while sliding against cartilage in different lubricant solutions.

– Effect of Sliding Duration

The effect of sliding duration was evaluated in BSA+HA+PRG4+POPC as a lubricant solution only.

A relative increase in COF with increasing the sliding duration was observed (**Figures 3B**, **5A,B** and **6B**). The maximum COF measured after 4 hours was 0.90 ± 0.44 on mPCU-s during swing. The average COF at the cartilage–meniscus interface during swing appeared to double, from 0.04 ± 0.02 after 1 hour to 0.09 ± 0.03 after 4 hours (**Figure 5A**). Nevertheless, the differences between 1-hour and 4-hour measurements during both stance and swing for each specific material were not significant (**Figure 5A,B**). The only exception was mPCU-s for which the COF during stance significantly increased (p=0.028) with increasing the sliding duration from 1 to 4 hours (**Figure 5B**).

Similar to the 1-hour experiments in experiments lasting 4 hours no significant differences were found between modified and unmodified PCUs with respect to the COFs measured while sliding against cartilage. The only exception was mPCU-s during stance (p = 0.048) (**Figure 5B**).

After 4 hours of sliding, all COFs measured during swing, at cartilage–PCU (all) interfaces were significantly higher than the ones measured for cartilage–meniscus interfaces (**Figure 5A**). During stance, on the other hand, the only significant

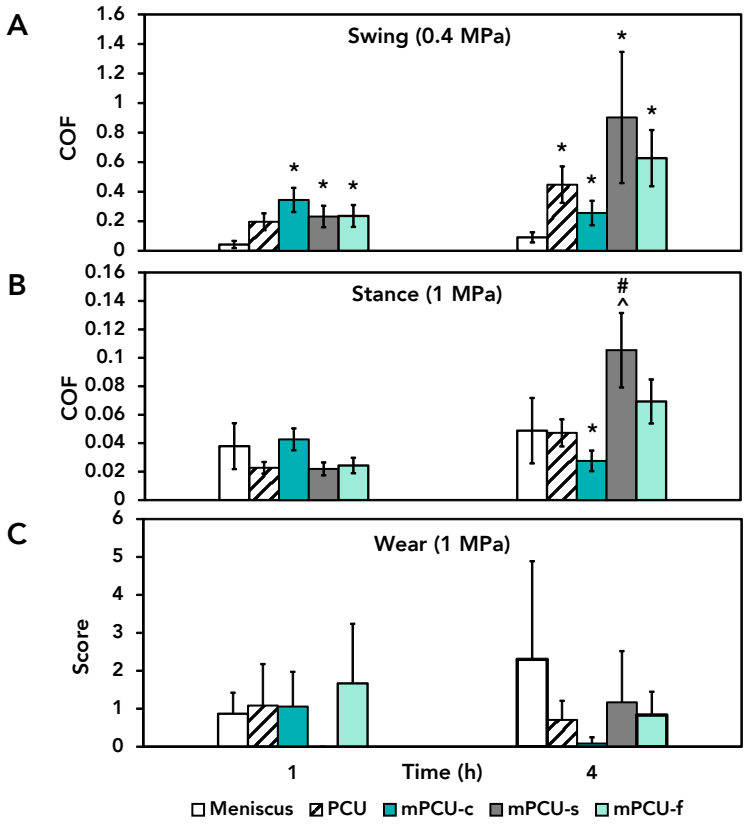


Figure 5. Average COF measured at different sliding duration during **A:** swing (0.4 MPa) and **B:** stance (1 MPa), in the presence of BSA+HA+PRG4+POPC in PBS for 1 hour or 4 hours (plots have different scales). Error bars indicate SD of the mean of COFs over 2–4 separate measurements. **C:** Scores of wear of cartilage at the end of the friction experiments reported in **A** and **B**. Error bars indicate SD of the mean of the scores. Significant differences ($p < 0.05$) are indicated by asterisks (*; as compared to meniscus in that specific solution), the hash sign (#; as compared to the same biomaterial in 1-hour experiment) and the caret (^; as compared to PCU in that specific solution).

difference (p = 0.029) was observed at the cartilage–mPCU-c interface, where a lower COF as compared to cartilage–meniscus was assessed (**Figure 5B**).

– Effect of Contact Pressure (P_c)

It is clear that the COFs during the stance phase were much lower than those measured during the swing phase (**Figures 3–5**). This identifies P_c as an important variable influencing the COF. The P_c of individual measurements was correlated to the measured COF (**Figure 6A**). Negative correlations were found for both cartilage–

PCU materials interface (blue circles) and cartilage–meniscus interface (black circles). Within the first 1 hour of the experiments and in the for $0.15 < P_c < 1.6$ MPa, a rough approximation of the COF can be made via a power equation 1, if the P_c is known.

$$COF = \alpha P_c^{\beta} \quad (1)$$

In this equation β is the contact pressure exponent and α is a multiplication factor, with values of −1.56 and 0.03 for cartilage–PCUs and −0.55 and 0.02 for cartilage–meniscus interface, respectively.

As the measurement time changed from 1 to 4 hours (filled black vs empty black circles), the COF increased during both stance and swing for cartilage–PCUs interface (**Figure 6B**). An equation of the form (1) can also be used to relate the COF to P_c for 4-hour experiments for cartilage–PCU materials interface (**Figure 6B**, dashed black line), in which β decreased very slightly to −1.58 and α increased to 0.06 (2 fold), compared to the blue line in **Figure 6A**. For cartilage–meniscus interface (**Figure 6C**), β increased to 0.08 and α increased to 0.07 (3.5 fold), compared to the black line in **Figure 6A**.

When the P_c increased from 1 to 4.5 MPa, during swing, at the cartilage–PCU interface, the COF increased significantly from 0.20 ± 0.06 to 0.49 ± 0.16 (2.5 fold) (**Figure 7A**); however, the COF at the cartilage–meniscus interface did not increase significantly. During stance, increasing the P_c to 4.5 MPa did not cause any further decrease in the COF, with an average COF smaller than 0.06, neither at the cartilage–PCU nor at the cartilage–meniscus interfaces (filled blue circles in **Figure 6B,C**).

When the sliding duration increased from 1 to 4 hours at 4.5 MPa for cartilage–PCU interface (**Figure 6B**, gray circles), the COF increased slightly with average values of 1.1 ± 0.5 and 0.08 ± 0.03 for swing and stance, respectively.

Wear

The damage to the cartilage surface of the osteochondral plugs was quantified at the microscopic level (**Figures 4C**, **5C** and **7C**). The scores varied between 0 and 2. No significant differences in the scores were found between cartilage that slid against meniscus and cartilage that slid against PCUs. Also, surface modification of PCUs, lubricant solution chemistry, sliding duration for 1 hour up to 4.5 MPa and 4 hours up to 1 MPa contact pressure did not result in differences in damage scores. Only the combination of 4-hour experiments at 4.5 MPa for cartilage–PCU interface increased the wear to 4.3 ± 1.2.

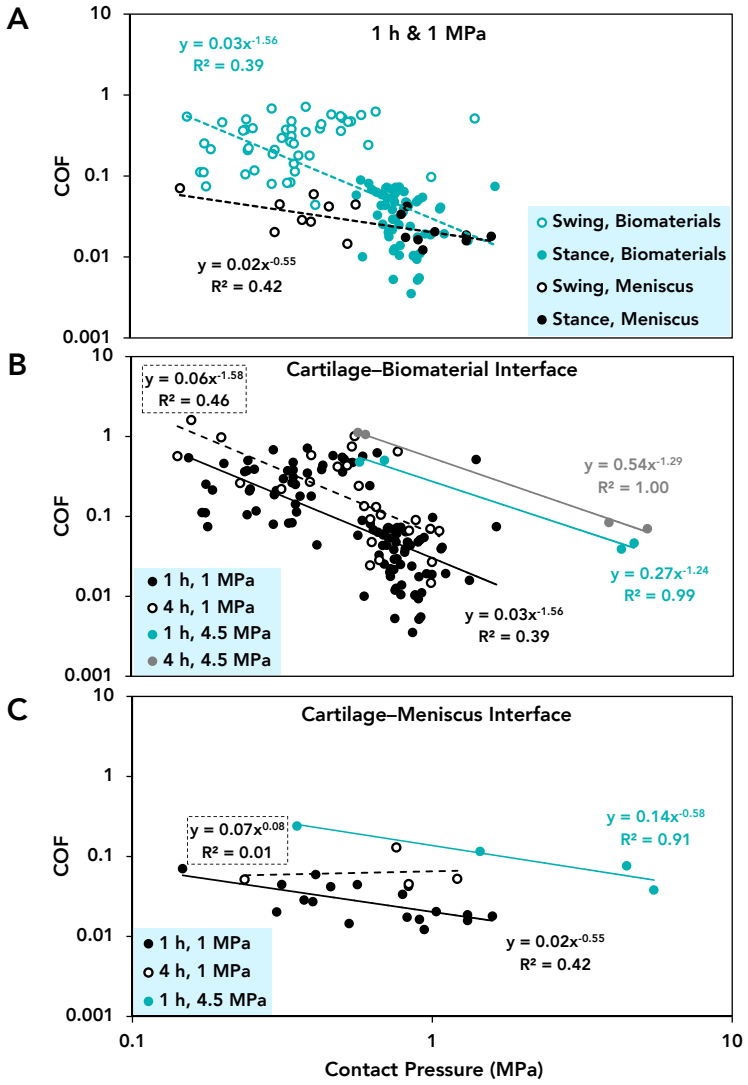


Figure 6. COF versus P_c plots measured: **A:** during the swing (0.4 MPa) (empty circles) and stance (1 MPa) (filled circles), at the cartilage–meniscus (black circles) and cartilage–PCU materials (blue circles) interfaces, irrespective of surface modifications, **B:** at the cartilage–PCU materials interface; filled black circles are related to all materials and all lubricant solutions; empty black circles are related to all materials tested in BSA+HA+PRG4+POPC; blue and gray circles are related to cartilage–PCU interface, in the presence of BSA+HA+PRG4+POPC and **C:** at the cartilage–meniscus interface, filled black circles are measured in the presence of all lubricant solutions; empty black and blue circles are measured in the presence of BSA+HA+PRG4+POPC.

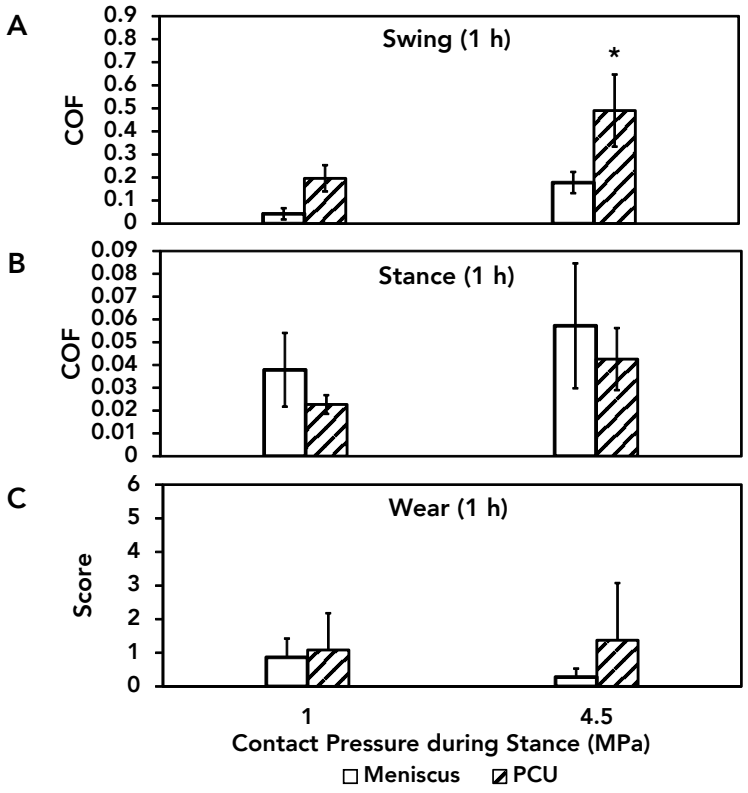


Figure 7. Average COF measured for different P_c's during stance at the cartilage–meniscus (white bars) and cartilage–PCU (hatched bars) interfaces, for 1 hour, in the presence of BSA+HA+PRG4+POPC, at the P_c of **A:** 0.4 MPa during swing and **B:** 1 and 4.5 MPa during stance (plots have different scales). Error bars indicate SD of the mean of COFs over 2–4 separate measurements. **C:** Scores of wear of cartilage at the end of the friction experiments reported in **A** and **B**. Error bars indicate SD of the mean of the scores. The asterisk (*) denotes significant differences ($p < 0.05$) as compared to 1 MPa.

Discussion

This work is the first *in vitro* study describing friction and wear at the cartilage–meniscus interface. The results are used to further explore the tribological changes occurring when meniscus tissue is replaced with a PCU biomaterial, mimicking replacement of meniscus with meniscus prosthesis in an otherwise healthy knee joint. We aimed at performing measurements at P_c's of 0.4, 1 and 4.5 MPa.[134] The effective average P_c values were 0.4 ± 0.2, 0.9 ± 0.2 and 4.6 ± 0.8 MPa. The measurements were performed for 1 and 4 hours at 1 Hz mimicking the same duration of continuous walking or 9 and 36 hours of normal activity, respectively, assuming

one million steps taken per year.

The COF at the cartilage–meniscus interface at 1 MPa after 36 hours of normal activity (0.05 ± 0.02) was twice the highest value (0.023) measured by Charnley in 1960 for a human knee joint with intact cartilage.[5] Cartilage and meniscus are porous tissues. When compressed at 1 MPa, the interstitial fluid weeps out of the pores and creates a thick fluid film at the interface according to the IFPW mechanism. This lubricates the sliding surfaces for hours, resulting in such low COF values. Conversely, during swing (at 0.4 MPa, after 36 hours of normal activity) the COF was 0.09 ± 0.03, almost 5 times higher than the maximum COF measured by Charnley. These high COFs indicate that at low P_c's not enough interstitial fluid weeps out to the interface to create a thick fluid film. On the other hand, the COF lower than 0.1 excludes pure boundary lubrication,[1] indicating a mixed IFPW and boundary lubrication mechanism to be active.

At the cartilage–meniscus interface, within the first 9 hours of normal activity, the COF decreased with increasing P_c according to power equation 2 (for $0.15 < P_c < 1.6$ MPa) (**Figure 6C**, filled black circles).

$$COF_{Cartilage-Meniscus} = 0.02\, P_c^{-0.55} \qquad (2)$$

Such a decrease in COF in a similar pressure range for cartilage against a hard surface or cartilage–cartilage interface has been reported before.[27] However, it appears that higher P_c's (as observed for 4.5 MPa in this study) do not further decrease the COF, i.e., a P_c of around 1 MPa completely harnesses the IFPW lubrication mechanism.[34,128] In order to confirm this statement, extra measurements were performed at a P_c of 2.4 ± 0.4 MPa, and the average COF measured for cartilage–meniscus was 0.04 ± 0.03, which was not lower than the average COF measured at 1 MPa (data not shown).

When meniscus tissue was replaced with PCUs, increased COFs were expected. This did occur during the swing phase, but did not occur during the stance phase.

During the stance phase COFs for cartilage–PCUs interface were lower or as low as cartilage–meniscus interface (the lowest COF measured was 0.0035), indicating that IFPW lubrication mechanism is still active at the cartilage–PCU materials interface, even though the bulk PCU material is not porous. Thus, during stance the cartilage surface would be well protected from wear against biomaterials by a thick fluid film, mainly derived from the cartilage itself. The reason for measuring much lower COF (0.0035) during stance at the cartilage–PCUs interface as compared to

cartilage–meniscus interface (**Figure 6A**) could be the difference in quality of PCU materials and meniscus used here. The polymer disks were homogeneously thick, plane-parallel, pressure molded and flat. Whereas the meniscus samples were manually sliced from the whole meniscus, where the top side was smooth and untouched but the lower side could be rough and in some cases having irregular thickness along the sliding direction.

During the swing phase of the gait cycle, the cartilage–PCUs interfaces revealed significantly higher COFs (10 fold) than cartilage–meniscus interfaces, with the highest COF being 0.71 as compared to 0.07. COF values close to 1 during swing indicate complete breakdown of the IFPW lubricating mechanism. Under these conditions boundary lubrication (complete contact between cartilage and biomaterial) must facilitate the sliding process. If so, the surface modifications of PCU were supposed to result in lower COF values, but that was not observed (**Figures 4A** and **5A**). A possible reason may be the presence of albumin in the lubricant solution. Albumin blocks the adsorption of PRG4, an important boundary lubricant, to the surface of the biomaterials.[131] Phospholipids, another important molecules in the joint lubrication, did not adsorb on the surface of the PCUs when brought in the form of vesicles (**Supplementary Data Figure 1**).

The COF at the cartilage–PCUs interface (for $0.16 < P_c < 1.6$ MPa) can be roughly estimated by power equation 3 (**Figure 6B**, filled black circles).

$$COF_{Cartilage-Meniscus} = 0.03\, P_c^{-1.56} \quad (3)$$

The sensitivity to P_c (β) is threefold higher than the one of cartilage–meniscus interface (Equation 2).

After 36 hours of normal activity, the COF of cartilage–PCUs interfaces during the swing phase even becomes 10 fold larger than at the cartilage–meniscus interface, with a maximum measured value of 1.6 (**Figures 3B** and **5A**).

The standard deviations in **Figures 4**, **5** and **7** and the spread in points shown in **Figure 6** indicate that besides P_c's other variables also affect the COFs. One would be the aspect ratio (AR) of the contact zone taken (**Figure 1G**). Ideally we expect a round circular contact area (AR = 1) between the cartilage and PCU materials/meniscus, but that was not always the case. We observed $1 < AR < 2$, caused by misalignment or lack of roundness of the femoral head. The AR appeared not to be related to COF (**Figure 8**). Thus, variability in data cannot be explained using AR.

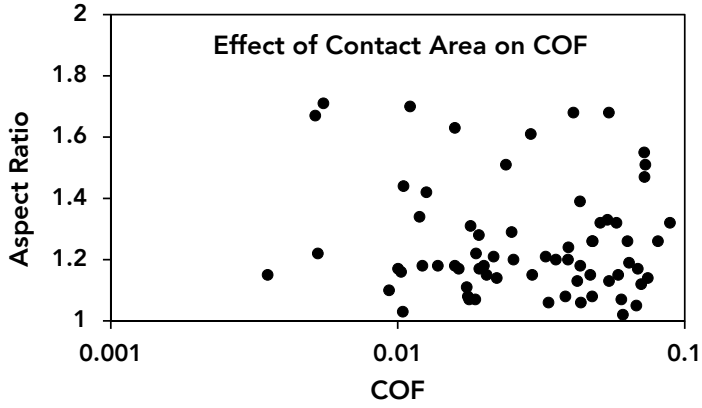


Figure 8. Correlation between aspect ratio and COF during stance (1 MPa), for 1 hour, of all the PCUs and meniscus, in all the solution chemistries.

In the present study, the cartilage–meniscus and cartilage–PCUs sliding did not result in differences in damage to the cartilage surface, independent of the duration of sliding or the applied contact pressures. This indicates that the PCU biomaterials appear suitable for replacement of an articulating surface in the knee joint, at least for a limited period of time. Systematic experiments are required to reveal how these materials will behave in the long term. A small number of pilot experiments showed an increase in cartilage wear at high contact pressures—in 60-hour experiments a P_c of 1 MPa during stance did not increase the cartilage wear but a P_c of 4.5 MPa did (**Figure 9**). Additionally, monitoring the tribological performance of these implants *in vivo* is required. *In vivo* experiments aiming at elucidating the effects of inflammatory factors and the foreign-body reaction are relevant as well.

Conclusion

The use of mimicking stance and swing phases of the gait cycle during friction measurement illustrated the role of IFPW lubrication and mixed IFPW and boundary lubrication mechanisms at the cartilage–meniscus interface. At the cartilage–meniscus interface very low friction ($0.01 < COF < 0.12$) and wear (scores < 1) were observed. When meniscus tissue was replaced with a PCU material, the IFPW remained active during stance, resulting in comparable COFs. During the swing phase approximately 10 fold higher COFs were found as compared to cartilage–meniscus interface because the IFPW mechanism fails and the boundary lubrication mechanism involving PRG4 and POPC was not activated. Since none of the

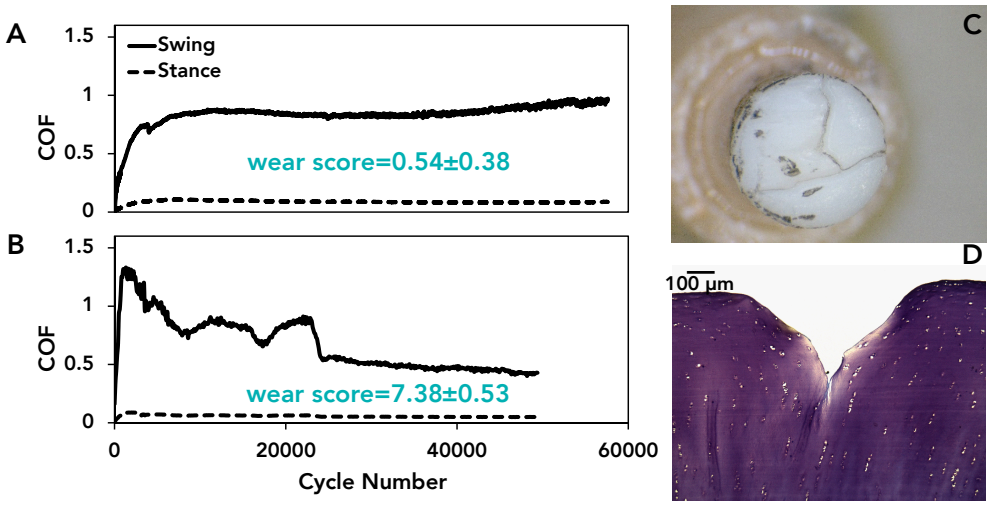


Figure 9. COF versus cycle numbers measured on PCU, for 60 hours, using UMT-3, at the contact pressure of 0.4 MPa during swing and **A:** 1 MPa and **B:** 4.5 MPa, during stance, in the presence of BSA+HA+PRG4+POPC. **C:** Damaged osteochondral plug at the end of the experiment at 4.5 MPa, related to plot **B**. **D:** The histology picture of the damage at the cartilage surface, related to plot **B**.

surface modifications of PCU was able to adsorb PRG4 and POPC, these lubricants remained ineffective in improving friction but there was a decrease in COF with increasing P_c up to ~1 MPa according to a power law, but any further increase in P_c does not decrease COF. Wear of cartilage against PCU materials was not higher than its wear against meniscus up to 36 hours at 1 MPa and for 9 hours up to 4.5 MPa.

Acknowledgments

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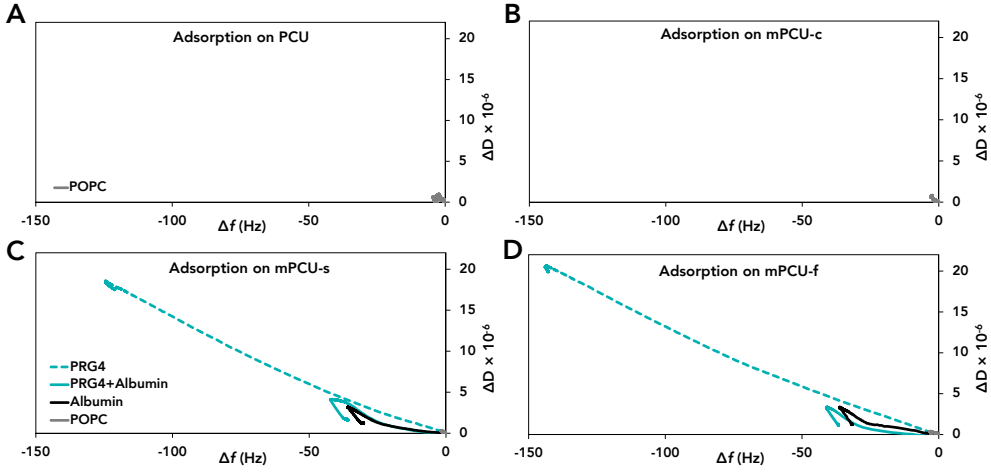
Supplementary Data

The adsorption mechanism of POPC vesicles on the PCU materials was investigated using quartz crystal microbalance with dissipation (QCM-D) with the same method explained elsewhere.[131] **Supplementary Data Figure 1A–D** shows that POPC vesicles (the gray curves) did not adsorb on any of the PCU materials. Furthermore, the adsorption mechanism of PRG4, albumin and PRG4+albumin on mPCU-s and mPCU-f was studied with the same method (explained elsewhere).[131] **Supplementary Data Figure 1C, D** shows that PRG4, albumin and PRG4+albumin have a similar adsorption trend as compared to PCU and mPCU-c (studied elsewhere[131]). PRG4 (dashed blue curves) adsorbed a lot and made a viscoelastic layer, as opposed to albumin (black curves), which adsorbed less and made a rigid layer on mPCU-s and mPCU-f. In a mixture (blue curves), albumin blocked PRG4 adsorption. The data for PRG4, albumin and PRG4+albumin adsorption on PCU and mPCU-c is not shown here to avoid repetition from **Chapter 3**.



Supplementary Data Figure 1. The dissipation shift versus frequency shift measured using QCM-D during the adsorption of PRG4, albumin, PRG4+albumin and POPC at **A:** PCU, **B:** mPCU-c, **C:** mPCU-s and **D:** mPCU-f.